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Keywords: fasting, postprandial, response, genome-wide association study, NMR-based metabolomics, single nucleotide polymorphism

Word count:
Abstract: 197

Figures/tables: 7 (figures=5, tables=2)

Supplemental figures/table: 10 supplemental tables and 2 supplemental figures

Main content: 4,937

Reference: 51

Abstract

Humans spend the greater part of the day in a postprandial state. However, the genetic basis of postprandial blood measures is relatively uncharted territory. We set out to examine the genetics of variation in concentrations of postprandial metabolites (t=150 min) in response to a liquid mixed meal through genome-wide association studies (GWAS) performed in the Netherlands Epidemiology of Obesity study (N=5,705). The metabolite response GWAS identified an association between glucose change and rs10830963:G in the melatonin receptor 1B (beta (SE): -0.23 (0.03), P-value: 2.15×10^{-19}). In addition, *ANKRD55* locus led by rs458741:C showed strong associations to extremely large VLDL particle (XXLVLDL) response (with XXLVLDLC: beta (SE): 0.17 (0.03) P-value: 5.76×10^{-10} and with XXLVLDLCE: beta (SE): 0.17 (0.03), P-value: 9.74×10^{-10}), which also revealed strong associations to body composition and diabetes in the UK Biobank (p-values$<5\times10^{-8}$). Furthermore, the associations between XXLVLDL response and insulinogenic index, HOMA-β, ISI matsuda index and HbA1c in the NEO study further implied the role of chylomicron synthesis in diabetes (with FDR corrected q-value<0.05). To conclude, genetic studies of metabolomics change after a liquid meal illuminate novel pathways for glucose and lipid metabolism. Further studies are warranted to corroborate biological pathways of *ANKRD55* locus underlying diabetes.

Introduction

Metabolites, the products of cellular activity and the environment, can be considered as intermediates between genes and clinical phenotypes (1). Recent developments in high-throughput metabolomic profiling based on mass spectrometry (MS) (1-4) and nuclear magnetic resonance (NMR) spectroscopy (2; 5-9) platforms have opened new avenues to explore gene-metabolite associations by genome-wide association studies (GWAS). In 2014, an atlas was generated describing 145 genetic loci associated with a broad spectrum of blood measures covering amino acids, carbohydrates, lipids, and peptides (10). A subsequent 2016 study, evaluating circulating fasting metabolites (mainly lipoprotein subclasses), observed over 60 genetic loci that were associated with at least one measure (11). These and other studies have provided considerable mechanistic insight into physiological pathways of diseases. However, the predominant focus of most studies has been on the genetics of metabolomic measures under fasting conditions.

Due to frequent food intake, humans spend the majority of their waking hours in a non-fasting state. However, insight in the genes that affect plasma metabolite concentrations in response to food intake is relatively limited. It seems more than likely that cumulative and prolonged exposure to multiple plasma metabolites in response to food intake may have pathological consequences, as has been well documented for certain lipids and lipoproteins (12-16). Further, it has long been observed that whilst there may be accepted relationships between consumption and metabolite abundance, there is also natural variation in this response (17).

In clinical practice, an oral-glucose tolerance test (OGTT) is commonly used for the screening of suspected diabetes and is performed by determining glucose levels two hours after ingestion of a fixed dose of glucose. Previous GWAS investigations have identified genetic loci that are associated with glucose and insulin responses from the OGTT (18), which only

partly overlapped with the GWAS findings on fasting glucose and insulin measures. The measurements from the OGTT have expanded the understanding of genetics and pathophysiology of diabetes. However, human food intake is more than sugar alone and similar to OGTT, meal responses reflected by metabolite profiles are likely to exhibit a large amount of variability, part of which will be heritable. A recent candidate gene study showed that the genetics of fasting and postprandial metabolite concentrations are overlapping (19). A recent twin study indicated that up to 48% of the variation in the two-hour glucose response to food can be attributed to genetics, whereas the genetic contribution to variation in the triglyceride and insulin responses was found to be nil to low (0% and 9%, respectively) (12).

In the current study, we aimed to further explore the genetics and heritability of plasma metabolomic measures as assessed by NMR spectroscopy before and after a liquid mixed meal. For this, we performed GWAS on fasting and postprandial metabolomic measure concentrations. We also performed GWAS on the response of these measures using different methods to assess these responses (the change from fasting state to postprandial state after a liquid mixed meal) in a large (N=5,705) population-based cohort study, the Netherlands Epidemiology of Obesity (NEO) study.

Materials and Methods

Study design and study population

This study was performed in a population-based prospective cohort, the Netherlands Epidemiology of Obesity (NEO) study (20). All 6,671 participants gave written informed consent and the Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved the study design. Initiated in 2008, the NEO study was designed to study pathways that lead to obesity-related diseases. Detailed information about the study design and data collection has been described elsewhere (20). Briefly, men and women aged between 45 and 65

years with a self-reported body mass index (BMI) of 27 kg/m^2 or higher living in the greater area of Leiden (in the west of the Netherlands) were eligible to participate in the NEO study. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited irrespective of their BMI.

Participants were invited for a baseline visit at the NEO study center in the LUMC after an overnight fast. Prior to their visits, participants completed a questionnaire at home with demographic, lifestyle and clinical data. At the baseline visit, fasting blood samples were drawn. Within the next five minutes after the fasting blood draw, a liquid mixed meal (400mL with 600 kcal, with 16 percent of energy (En%) derived from protein, 50 En% carbohydrates, and 34 En% fat) was consumed and subsequent blood samples were drawn 30 and 150 minutes after the liquid mixed meal. Individuals were excluded from the analyses (Figure 1) when 1) taking any lipid-lowering medication, 2) violating overnight fasting, 3) violating liquid mixed meal challenge protocol, i.e. did not finish the liquid mixed meal completely.

Genotyping and imputation

DNA was extracted from 6,671 venous blood samples obtained from the antecubital vein. Genotyping was performed in the Centre National de Génotypage (Evry Cedex, France), using Illumina HumanCoreExome-24 BeadChip (Illumina Inc., San Diego, California, United States of America). The detailed quality control process has been described previously (21). Genotypes were further imputed to the Haplotype Reference Consortium (HRC) release 1.1 (22). All genetic variants with an imputation quality below 0.4 or a minor allele frequency (MAF) below 0.01 were not considered for the analyses in the present study. As such a total of 5,705 individuals with genotype data for 7,381,632 variants were used in our association analysis.

NMR spectroscopy-based plasma metabolomics and other clinical chemistry measurements

Metabolomic measurements were performed in both fasting and postprandial (t=150 minutes after the liquid mixed meal) plasma samples using the Nightingale high-throughput NMR metabolomics platform (23). Each box of 94 samples (including both fasting and postprandial samples) had the samples run in the order that they were in the boxes. The sample order in the boxes was based on the sampling date, which guaranteed fasting and postprandial samples from the same participant being run in the same batch. Due to budget constraints and the fact that metabolite levels at 150 min change more significantly than 30 min after the meal (24), there were no metabolomic measurements at 30 minutes. We note that metabolites are commonly defined as biological molecules less than 1.5 kilo Daltons in size, and that many of the molecules (lipids) assayed here are larger than this threshold. Nevertheless, for simplicity we will refer to them all here as metabolites. The metabolomics platform provides measurements for 148 metabolites (Supplemental Table1) from eleven substance classes: lipoprotein subclasses (n=98), lipoprotein particle sizes (n=3), apolipoproteins (n=2), fatty acids and saturation (n=11), cholesterol (n=9), glycerides and phospholipids (n=9), amino acids (n=8), ketone bodies (n=2), inflammation (n=1), glycolysis related metabolites (n=3), and fluid balance (n=2). The NMR-based metabolomics platform and the experimental procedure have been described in detail previously (25).

In addition to metabolomics measurements, fasting glucose and insulin as well as postprandial glucose and insulin at 30 minutes and 150 minutes after a liquid meal were also measured by clinical chemistry approaches (26). Fasting hemoglobin A1c (HbA1c) concentrations were expressed in percentage (%). By using both fasting and postprandial measurements at 30 minutes and 150 minutes after the liquid mixed meal, insulin disposition

index (IDI), insulinogenic index (IGI), ISI matsuda index and HOMA index (i.e. HOMA-IR and HOMA-β) were derived based on standard formulas.

Sample and metabolite quality control and transformations

To remove samples of low quality and measurement errors, individuals were excluded when 1) metabolite concentrations deviated more than 10 standard deviation of the mean values from the entire NEO population and 2) it had more than 30% missingness across all 148 metabolites in either fasting or postprandial states (Figure 1). Metabolite concentrations were inverse rank normal transformed (27) using an edited version of the rntransform() function from the GenABEL package (28), which randomly ranks tied values. When analysing metabolites in the fasting or postprandial states alone each state was transformed independently of each other. However, when analysing or deriving the response phenotype the data from the two states were merged prior to data transformation. As it was previously observed that sampling date (a compound variable composed of blood sampling year-month) has an appreciable effect on metabolite concentration in this data set, we included this variable as a covariate prior to / during linear regression (29).

Defining metabolite responses to a liquid mixed meal

Metabolite response, or the change in concentration of a metabolite between the fasting and postprandial state was analysed in two manners. First and foremost, for each metabolite, we derived a response phenotype defined as the residuals of an orthogonal nonlinear least squares (OrNLSr) regression where the postprandial state was set as the dependent variable and the fasting state is the independent variable in a univariate analysis. The technical details regarding the OrNLSr method and comparison with other methods is discussed in the Supplemental Material. Response could have been defined as a simple estimation of change or a delta between postprandial and fasting states. However, we observed that for 30 of the 148 metabolites analysed, the data were best explained by a non-linear curve as opposed to a linear

one, possibly because a physiological plateauing effect for some metabolites (Supplemental Figure 1). A simple delta estimates of change or residuals derived from linear regressions between the two states would not accurately capture the variation of response, or metabolite change.

We also evaluated response via linear mixed models (LMM) that included an interaction term between dietary state (either fasting or postprandial state) and genotype alongside individual random effects. Under this framework, we simultaneous measured the effect of dietary state, genotype, and the interaction of state and genotype on metabolites. Here, the interaction term provides a measurement of genotype on response, or specifically a differential effect across the two states. Given the computational expense of the LMM, we chose to remove all genetic variants with an imputation quality below 0.4 or a minor allele frequency below 0.01 in the double-sized genotype data (N=11,410) before genome-wide association analysis for a specific metabolite, thereby also reducing our testing burden.

Genome-wide association analyses of metabolites under different prandial states

Four unique GWAS (fasting, postprandial, OrNLSr response, and LMM response) were performed on 148 metabolomic measures across 4734, 4348, 4292, and 11410 individuals, respectively (Figure 1), and 7,701,709 (7,568,622 for the LMM) genetic variants across the 22 autosomal chromosomes. For fasting, postprandial, and OrNLSr response, linear regression analyses, assuming an additive genetic model, were performed with SNPTESTv2 (30) on the residuals of inverse rank normal transformed metabolomic measures after adjusting for age, sex, the first ten principal components and the batch effect variable (a compound variable composed of blood sampling year-month). Considering that patients with diabetes are known to have a dramatically altered metabolism of macronutrients, we conducted sensitivity analyses by adding diabetes status as a covariate in the model and reran association analyses for top signals. The explained variance was estimated as the partial R^2 from the linear regression model,

with the SNP as independent variable and metabolite response represented by OrNLS residual (with inverse normal transformed) as dependent variable. For illustration purpose, we also reported the response to a liquid meal (i.e. OrNLSr response GWAS results) expressed as a percentage change from the fasting state. To obtain this percentage change, we reran the association analysis on the traits of interests using the raw OrNLS residuals (i.e. without inverse rank normal) divided by the baseline fasting measurements times 100, which gives the percentage change per allele. The p-value that comes out of this analysis is distorted due to non-normal distribution of phenotypes, but the effect size obtained corresponds to the value of a percentage change from the fasting state.

The LMM framework essentially tested for an interaction between genotype and dietary state. To reduce the computational burden, the linear mixed model was implemented in two steps. In the first step, fasting and postprandial metabolomic measures were concatenated into a long format phenotype file, and an edited version of inverse rank normal transformation, mentioned above, was applied to the raw measurements. After transformation, the linear mixed model with individual random effects was applied using the function lmer in the R package "lme4" (31), to the inverse rank normal transformed metabolite measures, further adjusting for age, sex, the first ten principal components and the batch effect variable sample date. Conditional residuals derived from the first step were subsequently used as dependent variables for linear regression models against genotype and dietary state interaction in the second step, performed by ProbABEL with sandwich standard error being estimated (32).

To identify independent loci in each metabolite-by-dietary state GWAS, we first identified linkage disequilibrium (LD)-independent blocks by clumping all variants with a standard GWAS significance level (alpha) of P-value$<5\times10^{-8}$ in PLINK (33). All genetic variants with a P-value below the alpha threshold ('-clump-p1') were set as "index" SNPs, and all the other SNPs were clustered into different clumps or LD blocks based on their linkage

disequilibrium (LD) and physical proximity to the "index" SNPs controlled by 'clump-r2' and 'clump-kb' separately in the command (4). In the current analyses, the following parameters were adopted: '--clump-p1' 5.0×10^{-8}, '--clump-r2' 0.5, '--clump-kb' 1000. Linkage disequilibrium patterns were based on the 1000 Genome v3 20101123 reference set of Utah Residents (CEPH) with Northern and Western European Ancestry (CEU) population (34).

In addition, primary and secondary signals were subsequently identified through step-wise conditional analyses using the genome-wide complex trait analysis (GCTA) tool version 1.24.4 (35), with a parameter of minor allele frequency (MAF) >0.01. A conditional P-value $<5\times10^{-8}$ was considered to be genome-wide significant. These primary and secondary signals identify tagging SNPs for an association and represent statistically independent (at a P-value$<5\times10^{-8}$) associations.

For each dietary state, we also defined "genomic regions" of association by collapsing all associated genetic variants that were within 500kb of each other into a single region. Finally, we compared the genomic regions defined for each dietary state to each other to define "study genomic regions". If multiple genomic regions, across dietary states, overlapped in their genomic coordinates they were merged into one region.

To account for the strong intercorrelations of lipoprotein subclasses (Supplemental Figure 2) in FDR based multiple test corrections, we applied the variance decomposition method proposed by Li et al (36). This resulted in 39/38/44 independent components underlying 148 metabolomic measures in the fasting state, the postprandial state, and the response as determined by OrNLSr respectively. Accordingly, metabolome-wide significance was set to 1.28×10^{-9}, 1.32×10^{-9}, 1.14×10^{-9} for fasting, postprandial and response GWAS (OrNLSr and LMM GWAS), respectively. Genome-wide significance was set to 5×10^{-8}, which was used to derive the metabolome-wide values by variance decomposition (36). Our study-wide alpha threshold is 3.0×10^{-10} (5×10^{-8} / (39+38+44+44)). The analysis workflow is shown in Figure 1.

Associations that surpassed the genome-wide alpha threshold were used to identify primary and secondary associations as well as to identify LD-independent blocks, and genomic regions of association.

Functional annotation of top SNPs

The independent top variants were entered in GTEx Portal (37), to identify the tissue-specific expression patterns (expression quantitative trait loci (eQTLs)) of mRNAs associated with these variants. The phenome wide association studies (Phewas) of independent top variants was performed by GeneAtlas based on 118 non-binary and 599 binary traits of 408,455 related and unrelated UK Biobank participants (38).

Estimates of heritability

Narrow-sense SNP-based heritability for each metabolomic measure under fasting, postprandial and OrNLSr derived response states was estimated by restricted maximum likelihood (GREML) under the framework of genome-wide complex trait analysis (GCTA). Genetic variants (both genotyped and imputed genotype data) with a minor allele frequency (MAF) >1% were retained for the analysis (32).

Data and resource availability statements

The individual-level datasets that are used for current study are not publicly available due to privacy issue. But all the summary statistics results from the genome-wide association studies are available from the corresponding author upon reasonable request. No applicable resources were generated or analyzed during the current study.

Results

Description of metabolites under different prandial states

Postprandial metabolite concentrations were correlated with their fasting levels (median absolute Pearson's r = 0.29, interquartile range [0.12, 0.59]). Notably, for 30 metabolomic measures, these associations were non-linear (Supplemental Figure 1). Since the effects of fasting state levels on the response was regressed out by OrNLSr regressions, the metabolite response residuals as determined by OrNLSr showed low correlations to either fasting (median absolute Pearson's r = 0.088, interquartile range [0.043, 0.14]) or postprandial (median absolute Pearson's r = 0.11, interquartile range [0.047, 0.19]) state measures.

Fasting GWAS

In total 32,212 SNP-fasting metabolite associations were discovered at a P-value less than 5×10^{-8} (Supplemental Table 2). These associations include 2249 unique genetic variants and 144 of the 148 tested metabolites. A total of 743 independent, primary associations were identified, which involve 119 unique SNPs and mapped to 39 genomic regions (Supplemental Table 2, Supplemental Figure 3). In addition, 16 secondary associations were identified involving 6 unique SNPs and 15 metabolites (Supplemental Table 3). The 16 secondary signals only surpassed the GWAS alpha threshold in the conditional analysis and were not among the initial 32,212 associations. In total 512 fasting primary associations surpass our study-wide alpha threshold of 3.0×10^{-10} (Supplemental Table 2).

Among the 62 associations previously reported by Kettunen et al. (11), 46 could be tested in the current fasting GWAS (the other 16 associations could not be evaluated due to our quality control: SNP MAF<0.01 or imputation quality <0.4). Among these 46 identified associations, 36 were successfully replicated (P-value < 0.05) in the NEO study (Supplemental Table 4).

Postprandial GWAS

At a P-value less than 5×10^{-8}, the postprandial metabolite GWAS identified 30,747 SNP-metabolite associations (Supplemental Table 5). These associations include 1817 unique genetic variants and 140 of the 148 tested metabolites. A total of 675 independent, primary associations were identified using joint conditional analysis, which involve 94 unique SNPs and mapped to 28 genomic regions (Supplemental Table 5; Supplemental Figure 4). In addition, 14 secondary associations were identified involving 8 tagging SNPs and 13 metabolites (Supplemental Table 3). In total 475 postprandial primary associations surpass our study-wide alpha threshold of 3.0×10^{-10} (Supplemental Table 5).

Response GWAS of residuals derived by OrNLSr

In the GWAS of residuals derived by OrNLSr, we identified 234 SNPs that contributed to variation in metabolite response to a meal (P-value $<5\times10^{-8}$, Supplemental Table 6). These associations include 74 unique genetic variants and 23 of the 148 tested metabolites. A total of 23 primary associations were identified using joint conditional analysis, which involve 14 unique SNPs and mapped to 12 genomic regions (Supplemental Table 6; Supplemental Figure 5). No secondary associations were observed.

Three primary associations surpassed the metabolome-wide alpha in the OrNLSr GWASs and map to two genomic regions (Table 1). The strongest association, which also surpasses our study-wide alpha, is on chromosome 11, is tagged by rs10830963:G (MAF: 0.26, beta (SE): -0.23 (0.03), P-value: 2.15×10^{-19}) at the *MTNR1B* locus (Figure 3a and Table 1) and is associated with glucose response. rs10830963 explained 1.9% of total variance in glucose response represented by OrNLS residuals. In addition, rs458741:C located on chromosome 5 (*ANKRD55* locus) was associated with extremely large VLDL total cholesterol levels (XXLVLDLC) (MAF: 0.23, beta (SE): 0.17 (0.03), P-value: 5.76×10^{-10}) and extremely large VLDL cholesterol esters levels (XXLVLDLCE) (MAF: 0.23, beta (SE): 0.17 (0.03), P-value:

9.74×10^{-10}), respectively. Although did not reach study-wise significance, signals led by rs467022:T and rs173964:G at the *ANKRD55* locus also showed suggestive associations to XXLVLDLL, XXLVLDLP, XXLVLDLTG, XXLVLDLFC, XXLVLDLPL (Table 1). The three SNPs (i.e. rs458741, rs467022 and rs173964) located in ANKRD55 locus in total explained 0.85-0.98% of total variance of seven XXLVLDL-related metabolite response

Response effects by linear mixed model analysis

To further assess whether genetic variants associated with fasting or postprandial metabolomic measure concentrations affected response, we performed a genome-wide linear mixed model analysis for each metabolite in an alternative assessment of meal response. We identified a total of 37 genetic variants that contributed to variation in metabolite response to a meal in this mixed model analysis (P-value $<5\times10^{-8}$, Supplemental Table 7). These associations include 15 metabolites and 31 SNPs (15 unique SNPs) mapping to 12 genomic regions (Table 2). The strongest effects were observed for XLHDLL and citrate, mapping to rs116041093 and rs632200, respectively (Table 2). Both of these genetic variants had a low effect allele frequency (0.03 and 0.01 separately) and did not reach genome-wide significance in the OrNLSr response GWAS. The third strongest effect was the glucose response and mapped to rs10830963:G in the intron of *MTNR1B* gene (genotype-by-dietary state interaction: beta (SE): -0.16 (0.02), P-value: 4.66×10^{-9}), which is consistent with findings in the OrNLSr response GWAS (Supplemental Figure 6). The sensitivity analysis by adding diabetes status as a covariate showed very similar effect size estimations (data not shown).

Overlap in associations among dietary states

The fasting and postprandial GWASs shared an abundance of overlap. In total 86% (26,419 of the 30,747) of the postprandial associations were also observed among the fasting associations. Moreover, 397 of the 675 independent primary SNP-metabolite postprandial associations were also primary associations in the fasting data set. This overlap includes 131

metabolites and 50 SNPs, the most common of which are SNP rs964184 at band 11q23.3 near the *APOA5* locus (n = 48 metabolites), previously associated with hypertriglyceridemia (39), and the *APOE* exonic missense SNP rs429358 at band 19q13.32 (n = 59 metabolites) previously associated with hyperlipoproteinemia (40) and Alzheimer's disease (41; 42). In contrast, 278 primary association were unique to the postprandial state, while 346 were unique to the fasting dietary state.

However, if we look not at overlap in tagging SNPs but the genomic loci they map to, we find that there are 22 genomic regions involving 139 metabolites that carry independent, primary associations (n = 546 genomic region, metabolite pairs) in both the fasting and postprandial state (Supplemental Figure 7, Supplemental Table 8). In contrast 15, 5, and 10 genomic regions harbour SNP-metabolite primary associations in just one dietary state – fasting, postprandial, and response, respectively. This includes the examples: glucose at (1) the *G6PC2* locus (rs560887, band 2q31.1, Study-GR8), at (2) the *AGMO* locus (rs10231021, band 7p21.2, Study-GR20), at (3) the *GCK* locus (rs2971670, band 7p13, Study-GR21), and at (4) the *MTNR1B* locus (rs10830963, band 11q14.3, Study-GR31), which each exhibit an association with glucose in the fasting state (P-value< 2.20×10^{-8}) but not in the postprandial state (P-value> 0.0037).

Functional annotation of rs10830963 and examination of glucose response in the NEO cohort

A previous study showed that rs10830963 is an eQTL in human islets conferring increased *MTNR1B* mRNA expression. The G-allele carriers exhibited a stronger inhibition of insulin secretion after melatonin treatment (43). Given the consistently observed association between rs10830963:G and glucose response from our metabolite response GWAS, glucose as measured by a clinical chemistry laboratory at fasting state, 30 minutes and 150 minutes after a liquid mixed meal were examined in the NEO cohort stratified by rs10830963:G genotype.

The postprandial glucose excursions, defined as the change in glucose concentrations from before to after a meal, showed evidence of difference (fasting status and genotype interaction p-value: 7.6×10^{-4}) (Figure 3b). rs10830963:G has been linked to several glycaemic traits in previous large-scale GWAS meta-analysis, with the strongest signals on fasting glucose (Figure 3c), followed by HOMA-β (for beta-cell function).

Pathophysiological insights from novel chylomicrons and extremely large VLDL particle response associations

The three SNPs (namely rs458741, rs467022 and rs173964) are in high linkage disequilibrium (LD) with each other and belong to the same *ANKRD55* locus. By a Phewas in the UKBiobank, the *ANKRD55* locus is significantly associated with several traits reflecting body composition (e.g., impedance of arms and legs, waist-to-hip ratio, trunk fat percentage and body fat percentage), blood traits (e.g., red blood cell distribution width, platelet distribution width and haemoglobin concentration) and diabetes, with p-values$<5\times10^{-8}$ (Figure 5a). Except for rs458741without eQTL information, rs467022 and rs173964 both are eQTLs in multiple tissues conferring increased mRNA expression levels, with the strongest expression levels in kidney (for rs467022) and esophagus as well as whole blood (for rs173964) separately (Figure 4a). A previous study performed by Scott et al. has reported a T2D association signal led by rs173964 at the *ANKRD55* locus, with an odds ratio of 1.08 (44), however, we did not replicate this association in the NEO study (T2D cases/controls: 254/4215), which is likely due to the lack of power. To further understand the roles of chylomicrons and extremely large VLDL particle response in the risk of T2D, we investigated the associations between responses of XXLVLDL particles (i.e. rXXLVLDL) and T2D risks as well as other glycaemic traits in the NEO study. Although there is no significant association to T2D, we identified strong links between the responses of chylomicrons and extremely large VLDL particle (i.e., rXXLVLDL)

to insulinogenic index (IGI), insulin sensitivity (ISI) Matsuda index, HOMA-β and HbA1c (Figure 4b and Supplemental Table 10), after adjusting for age, sex and BMI.

Heritability

On average, the SNP heritability was 31% for fasting metabolomic measure concentrations, which was higher than the average SNP heritability for postprandial metabolomic measures concentrations and response measures (27% and 12%, respectively) (Supplemental Table 9). SNP heritability for fasting state metabolomic measures was higher than the counterpart postprandial measures (paired one-sided Wilcoxon-Rank test, p-value=2.2×10^{-11}). Overall, the heritability of metabolomic measure responses was much lower than either fasting or postprandial state measures. However, the response measures for glucose, the amino acids (except for histidine), XXLVLDL, and XLHDL showed a heritability estimate of approximately 25% (Figure 5). In fact, heritability point estimates for glucose (0.27, SE=0.07), alanine (0.29, SE=0.08), phenylalanine (0.22, SE=0.07), valine (0.31, SE=0.07) and XXLVLDL traits (mean=0.28) were larger in the response state than they were in fasting and postprandial states.

Discussion

By performing GWAS on measures before and after a liquid mixed meal and on parameters describing meal response. We observed highly overlapping genetic association signals between fasting and postprandial metabolites. By using baseline-adjusted nonlinear residuals to determine the meal response, rs10830963:G, located in the intron of gene *MTNR1B*, was observed to be associated with glucose response, which was also found using a linear mixed model based approach. Importantly, we identified a previously unreported distinct association between *ANKRD55* locus led by rs458741, rs467022 as well as rs173964 and extremely large VLDL particle response (all seven XXLVLDL particles). The heritability of the meal response measured by OrNLSr was much lower, on average, than either fasting or postprandial state measures. This suggests that variation in the meal response is to a larger extent more attributable (or susceptible) to non-genetic determinants, such as environmental exposures like the gut microbiome (45).

The contribution of liquid mixed meal to understand carbohydrate metabolic pathways

MTNR1B plays a role in central circadian clock regulation to accommodate diurnal rhythms (46). rs10830963:G is located in the middle of the single intron of *MTNR1B* gene was found to be associated with fasting glucose levels and type 2 diabetes risk in previous GWAS studies (47; 48). Concurrently, this variant was shown to be associated with decreased early-phase insulin secretion (49; 50), which is normally considered as the earliest detectable abnormality in individuals that are prone to develop type 2 diabetes (51). In line with this, G-allele carriers were observed to have a 20% increased risk to develop pre-diabetes (hazard ratio [HR] (95% confidence interval [95%CI]: 1.20 [1.15, 1.27]), whereas no additional risk was observed for the progression to type 2 diabetes from an impaired fasting glucose state (HR [95%CI]: 0.98 [0.89, 1.07]) (52). In the current study, glucose response was induced by a liquid

mixed meal with 50% energy from carbohydrates, which more closely resembles regular meal intake. We observed a strong effect of the rs10830963:G risk allele on plasma glucose levels at 30 minutes after a meal (Figure 3b). Theoretically, this translates to a life-long accumulative effect from this genotype on glucose exposure after meals and likely plays an important role in the development of glucose intolerance and insulin resistance. A recent study showed that glycemic response to a meal for the same individuals was on average twofold higher at lunch than breakfast (45). Taking the fact of meal timing into account, the effect of rs10830963:G risk allele on glucose response identified in the current study would be even more striking, as the meal challenge intervention was performed between 8 am and 12 pm (i.e. before lunch) in the NEO study.

The contribution of liquid mixed meal to understand lipid metabolism

The liquid mixed meal used in the current study provided 34% energy from fat, which confers an opportunity to investigate the exogenous pathway for lipid metabolism. Dietary cholesterol and fatty acids are absorbed to small intestine, at where micelles are formed and subsequently transported to enterocyte. Triglycerides (TGs) are formed from free fatty acids and glycerol, and cholesterol is esterified. Inside the Golgi body, TGs combine with proteins (e.g. ApoB-48) synthesized in rough endoplasmic reticulum (ER) to form chylomicrons (Figure 5b), which enter the circulation and travel to peripheral sites. *ANKRD55* locus led by rs458741, rs467022 and rs173964 showed strong associations to the responses of chylomicrons and extremely large VLDL particles in the blood, implying its role in chylomicron synthesis and transportation. Interestingly, *ANKRD55* locus is associated with both body composition and diabetes. The association between rs173964 and the risk of T2D has been reported previously by Scott et al (44), however, the function of rs173964 was not elucidated in the article. By deriving endophenotypes of chylomicrons and extremely large VLDL particle responses to a liquid meal, we observe that the responses of chylomicrons and extremely large VLDL particles

are strongly associated with glycaemic indicators for β-cell function (i.e. insulinogenic index and HOMA-β), insulin resistance (i.e. ISI matsuda index) and a surrogate for the average blood glucose levels in the previous 3 months (i.e. HbA1c). In summary, *ANKRD55* locus could potentially affect chylomicron synthesis and transportation after fat intake, and consequently influence beta-cell function and lead to a higher risk of T2D ultimately.

The implications to personalised nutrition

Personalised nutrition aims to exploit the interactions between genetics and modifiable environmental factors to determine food choices that lead to favourable glycemic and lipidemic responses and presumably decreased disease risk. Predictors from the gut microbiome, genetic variants, habitual diet and meal context have explored in prediction models of glycemic responses (45; 53) and lipidemic responses (i.e. triglyceride response) to different types of food (45). It was recently shown that the genetic component explained nearly half of the variation in the glucose response, however, the influence on triglyceride and insulin response (reflected by C-peptide) was minimal (0% and 9% separately). In the current study, we found that the SNP-based heritability of the glucose response to a meal was 27% (SD: 7.2%), whereas only 7% (SD: 7.4%) of variation in the serum triglyceride response was due to genetics. Our findings are in line with the previous study and in addition, we extended the heritability estimations of a meal response to a spectrum of 148 metabolomic measures. In general, we found that genetics was not a predominant determinant of meal response for most metabolites (on average 12%). This clearly challenges the application of genetics for personalized nutrition advice.

Strengths and limitations

Several methodological aspects should be considered. The main strength of this study is the liquid mixed meal that was provided to all the NEO participants, which more closely resembles normal meal consumption than a glucose tolerance test to assess glucose metabolism

after a meal. We generated novel insight in the genetic basis for fasting and postprandial metabolite concentrations in a general population. Moreover, to assess metabolite responses, we used two different methods to account for the potential bias introduced by baseline adjustment. When genetics contributions to metabolite responses after a meal are assessed, it is important to realize that these responses are affected by their fasting baseline abundance. In the original analysis plan, we adopted plain delta (i.e. the difference between postprandial and fasting measures) to represent metabolomic responses, as several other GWAS efforts on response measures (26; 54). However, once the theoretical framework of the OrNLS method had been fully developed, we were convinced that we also needed to employ it in our present study, because OrNLS corrects the metabolite response measure much better for the baseline values than plain delta and an LMM given that it takes nonlinear response profiles into account. Since LMM is widely considered as the de facto statistical approach to analyse repeated measures data, even though we believe it has certain shortcomings in the setting of our present study (e.g. LMM cannot address the non-linear relationships between fasting and postprandial measurements that we observed in our data), we decided to still include the results from the LMM as a reference. Nonetheless, the sample size of the genome-wide association study was relatively small to identify low frequency and rare genetic variants. In addition, it is not known whether the association between rs10830963:G genotype and glucose response is generalizable to different time periods of the day. Last but not least, due to the uniqueness of the current study design administering all participants a liquid mixed meal, we could not replicate our findings in other independent cohorts, which calls for further efforts to investigate our findings.

Conclusions

The majority of metabolomic response variation does not have a large genetic component. This does raise questions regarding the applicability of genetic variants to predict postprandial responses to food. We observed that the rs10830963:G variant in the *MTNR1B* gene is a genetic

determinant of both fasting glucose levels as well as the fasting-independent postprandial glucose response to a liquid mixed meal. *ANKRD55* locus led by rs458741, rs467022 and rs173964 showed strong associations to extremely large VLDL particle response, which were linked to beta-cell function and insulin resistance. Further studies are warranted to corroborate biological pathways of *ANKRD55* locus underlying diabetes.

Acknowledgements

The authors thank all individuals who participated in the Netherlands Epidemiology of Obesity study, and all participating general practitioners for inviting eligible participants. We also thank the NEO study group, Pat van Beelen and all research nurses for data collection, Petra Noordijk and her team for laboratory management, and Ingeborg de Jonge for data management of the NEO study. We acknowledge the support from the Netherlands Cardiovascular Research Initiative: an initiative with support of the Dutch Heart Foundation (CVON2014-02 ENERGISE). We thank Nutricia Research, Utrecht, The Netherlands, for providing the mixed meal. Nicholas J. Timpson is a Wellcome Trust Investigator (202802/Z/16/Z), is the PI of the Avon Longitudinal Study of Parents and Children (MRC & WT 102215/2/13/2), is supported by the University of Bristol NIHR Biomedical Research Centre (BRC-1215-20011), the MRC Integrative Epidemiology Unit (MC_UU_12013/3) and works within the CRUK Integrative Cancer Epidemiology Programme (C18281/A19169). David A. Hughes is supported by the Wellcome Investigator Award (202802/Z/16/Z). Dennis Mook-Kanamori is supported by Dutch Science Organization (ZonMW-VENI Grant 916.14.023).

Ethnics, consent and permissions

All 6,671 participants gave written informed consent and the Medical Ethical Committee of the Leiden University Medical Center (LUMC) approved the study design.

Funding

The NEO study is supported by the Netherlands Cardiovascular Research Initiative: an initiative with support of the Dutch Heart Foundation (CVON2014-02 ENERGISE). Dennis Mook-Kanamori is supported by Dutch Science Organization (ZonMW-VENI Grant 916.14.023).

Duality of Interest

Ruifang Li-Gao is a part-time consultant for Metabolon, Inc. All other authors have nothing to disclose.

Author Contributions

R. Li-Gao and D.A. Hughes performed the analysis and wrote and edited the manuscript. J.B. van Klinken derived the OrNLS response measurements. R. de Mutsert and F.R. Rosendaal contributed to study design and manuscript review. K. Willems van Dijk, D.O. Mook-Kanamori and N.J. Timpson contributed to study design, conceive the idea of the current study, interpret the results and review the manuscript.

Guarantors

R.Li-Gao is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation

Parts of this study were presented in abstract form at American Society of Human Genetics conference taken place on October 15-19, 2019 at Houston, the US.

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Table Legends

Table 1. Primary signals by nonlinear residual metabolite responses to a liquid mixed meal.

Table 2. Primary signals of meal responses to a liquid mixed meal by linear mixed model (LMM).

Figure Legends

Figure 1. Analysis workflow.

Figure 2. Miami plot of 148 fasting (top) and postprandial (bottom) metabolites.

Figure 3. Combinatorial plot of glucose response signals identified from GWAS. (a). Regional plot for the lead signal rs10830963; (b). An interaction plot of clinical chemistry glucose levels at fasting, 30 minutes and 150 minutes after a liquid mixed meal in the NEO cohort, stratified by rs10830963 genotype; (c). The -log10(p-values) of associations between rs10830963 and several glycaemic traits from literature; FG: fasting glucose; BW: birth weight; HbA1c: Hemoglobin A1C (HbA1c) test; T2D: type 2 diabetes; IResadjISI: corrected insulin response adjusted for insulin sensitivity index; IDI: Insulin disposition index; HOMA-B: the Homeostasis Model Assessment (HOMA) estimates steady state beta cell function (%B).

Figure 4. Pathophysiological insights from novel chylomicrons and extremely large VLDL particle associations. (a). The eQTL of three SNPs (rs458741, rs467022, rs173964) located in the *ANKRD55* locus that are associated with chylomicrons and extremely large VLDL particle response to a liquid mixed meal (response[r]XXLVLDLC, rXXLVLDLCE, rXXLVLDLL, rXXLVLDLP, rXXLVLDLTG, rXXLVLDLFC, rXXLVLDLPL), and significant traits that are associated with three SNPs located in the *ANKRD55* locus by phenome wide association studies (Phewas) in the UKBiobank. (b). The associations between rs173964 and type 2 diabetes as well as insulin sensitivity index in the NEO study and from literature, combined with an illustrations of lipid absorption and transport. ER: endoplasmic reticulum.

Figure 5. Heritability of 148 metabolites on different fasting status.

Table 1. Primary signals by nonlinear residual metabolite responses to a liquid mixed meal.

Metabolite	SNP	Chr	POS	Gene***	EA/ NEA	EAF	OrNLSr response					Fasting			Postprandial			LMM GWAS		
							beta	se	beta[§]	se[§]	p-value	beta	se	p-value	beta	se	p-value	beta	se	p-value
Metabolome-wide signals																				
Glc*	rs10830963	11	92708710	*MTNR1B*, intronic	G/C	0.26	-0.23	0.03	-0.23	0.03	2.15E-19	0.19	0.02	1.85E-15	-0.02	0.03	3.78E-01	-0.16	0.02	4.66E-09
XXLVLDLC*	rs458741	5	55807837	*ANKRD5*, intronic	C/T	0.77	0.17	0.03	0.10	0.03	5.76E-10	-0.02	0.03	4.75E-01	0.04	0.03	8.64E-02	0.03	0.01	3.77E-02
XXLVLDLCE*	rs458741	5	55807837		C/T	0.77	0.17	0.03	0.04	0.03	9.74E-10	-0.03	0.03	1.95E-01	0.04	0.03	1.35E-01	0.03	0.01	1.88E-02
Genome-wide signals																				
XXLVLDLL*	rs467022	5	55805639	*ANKRD5*, intronic	T/C	0.77	0.17	0.03	0.13	0.03	1.73E-09	-0.01	0.03	6.86E-01	0.05	0.03	5.99E-02	0.02	0.01	1.06E-01
XXLVLDLP*	rs467022	5	55805639		T/C	0.77	0.17	0.03	0.14	0.03	2.11E-09	-0.01	0.03	6.90E-01	0.05	0.03	5.88E-02	0.02	0.01	6.62E-02
XXLVLDLTG*	rs467022	5	55805639		T/C	0.77	0.17	0.03	0.14	0.03	2.58E-09	-0.01	0.03	7.38E-01	0.05	0.03	5.65E-02	0.02	0.01	8.37E-02
XXLVLDLFC*	rs173964	5	55809465	*ANKRD55*, intronic	G/A	0.78	0.16	0.03	0.05	0.03	4.26E-09	-0.01	0.03	7.47E-01	0.05	0.03	7.58E-02	0.02	0.01	8.68E-02
XXLVLDLPL*	rs173964	5	55809465		G/A	0.78	0.16	0.03	0.10	0.03	5.32E-09	-0.01	0.03	7.33E-01	0.05	0.03	8.00E-02	0.02	0.01	1.56E-01
DHA*	rs143754716	11	92131010	*FAT3*, intronic	G/C	0.02	0.54	0.09	0.51	0.09	7.00E-09	-0.05	0.09	5.59E-01	0.17	0.09	7.07E-02	0.25	0.04	1.91E-07
XLVLDLCE*	rs467022	5	55805639	*ANKRD5*, intronic	T/C	0.77	0.16	0.03	0.06	0.03	7.49E-09	0.01	0.03	5.68E-01	0.04	0.03	1.29E-01	0.03	0.01	4.82E-03
His*	rs7982187	13	27470380	*GPR12*, intergenic	G/T	0.24	0.15	0.03	0.14	0.03	9.80E-09	-0.08	0.02	1.16E-03	0.05	0.03	3.75E-02	0.12	0.02	1.43E-05
XLVLDLC*	rs173964	5	55809465	*ANKRD5*, intronic	G/A	0.77	0.16	0.03	0.05	0.03	1.11E-08	0.01	0.03	6.19E-01	0.03	0.03	1.83E-01	0.02	0.01	1.29E-01
SHDLFC*	rs114652642	5	105906380	*EFNA5*	C/A	0.03	-0.43	0.07	-0.39	0.08	1.23E-08	0.24	0.07	7.21E-04	0.09	0.07	2.38E-01	-0.18	0.03	1.43E-05
XLVLDLFC*	rs173964	5	55809465	*ANKRD5*, intronic	G/A	0.77	0.15	0.03	0.01	0.03	2.00E-08	0.02	0.03	5.22E-01	0.04	0.03	1.46E-01	0.03	0.01	2.60E-03
FreeC*	rs143642501	16	82387230	*MPHOSPH6*, intergenic	T/C	0.02	0.64	0.11	0.62	0.12	2.07E-08	-0.20	0.11	6.59E-02	0.13	0.12	2.63E-01	0.31	0.05	7.48E-07
SLDLCE*	rs28855728	4	31252214	*PCDH7*, intergenic	A/G	0.14	-0.17	0.03	-0.14	0.03	2.66E-08	0.01	0.03	8.65E-01	-0.03	0.03	2.76E-01	-0.06	0.01	3.93E-05
LDLTG*	rs2037053	12	101646796	*UTP20*, intergenic	G/C	0.04	-0.33	0.06	-0.34	0.06	3.74E-08	0.06	0.06	3.19E-01	-0.07	0.06	2.59E-01	-0.11	0.02	4.77E-04
SLDLC*	rs28855728	4	31252214	*PCDH7*, intergenic	A/G	0.14	-0.17	0.03	-0.14	0.03	4.18E-08	0.01	0.03	8.15E-01	-0.03	0.03	3.33E-01	-0.05	0.01	1.15E-04
MLDLTG*	rs2037053	12	101646796	*UTP20*, intergenic	G/C	0.04	-0.32	0.06	-0.32	0.06	4.20E-08	0.06	0.06	2.63E-01	-0.07	0.06	2.12E-01	-0.11	0.03	1.96E-03
SHDLFC*	rs150441681	13	100184791	*TM9SF2*, intronic	G/A	0.04	0.32	0.06	0.32	0.06	4.30E-08	-0.03	0.06	5.40E-01	0.10	0.06	9.79E-02	0.09	0.02	3.37E-03
XLHDLFC*	rs116717021	5	168758491	*SLIT3*, regulatory region variant	T/C	0.03	-0.40	0.07	-0.28	0.07	4.44E-08	0.05	0.07	4.17E-01	-0.04	0.07	5.71E-01	-0.12	0.02	1.19E-05
IDLTG*	rs118039629	12	101803806	*ARL1*, upstream gene variant	A/G	0.02	-0.44	0.08	-0.44	0.08	4.78E-08	0.03	0.08	7.00E-01	-0.13	0.08	1.06E-01	-0.12	0.03	9.07E-04
IDLTG*	rs71473282	10	88666064	*SNCG*, intronic	A/G	0.02	-0.40	0.07	-0.45	0.07	4.86E-08	0.08	0.07	2.84E-01	-0.04	0.07	5.50E-01	-0.14	0.03	7.39E-05

SNP: single nucleotide polymorphism; Chr: chromosome; EA/NEA: effect allele (coding allele)/non-effect allele (non-coding) allele; EAF: effect allele frequency/coding allele frequency; beta: effect size per coding allele; se: standard error.
* All secondary signals that were identified by step-wise conditional analysis in GCTA were highlighted with a star in the first column called Metabolite.
** The SNP was removed due to minor allele frequency < 0.01 in the genotype file before statistical analysis by linear mixed model.
*** Nearest coding gene with Ensembl Variant Effect Predictor (VEP).

§ the response to a liquid meal expressed as % change from the fasting state, calculated by using the raw OrNLS residuals (i.e. without inverse rank normal) divided by the baseline fasting measurements times 100, which gives the % change per allele.

Table 2. Primary signals of meal responses to a liquid mixed meal by linear mixed model (LMM).

Metabolite	SNP	Chr	POS	Gene	EA/ NEA	EAF	LMM GWAS					Fasting			Postprandial			OrNLSr response		
							beta	se	beta§	se§	p-value	beta	se	p-value	beta	se	p-value	beta	se	p-value
Metabolome-wide signals																				
XLHDLL	rs116041093	10	61264476	*FAM13C, intergenic*	A/G	0.03	-0.14	0.02	-0.31	0.08	1.50E-10	0.05	0.08	5.41E-01	-0.05	0.08	5.73E-01	-0.31	0.08	1.44E-04
Cit	rs632200	9	38603188	*ANKRD18A, missense*	T/G	0.01	-0.60	0.08	-0.23	0.10	1.70E-10	0.18	0.10	5.51E-02	-0.12	0.10	2.28E-01	-0.30	0.10	3.69E-03
Genome-wide signals																				
Glc	rs10830963	11	92708710	*MTNR1B*, intronic	C/G	0.26	-0.16	0.02	-0.23	0.03	4.66E-09	0.19	0.02	1.85E-15	-0.02	0.03	3.78E-01	-0.23	0.03	2.15E-19
XLHDLC	rs116041093	10	61264476	LOC107984235, intronic	A/G	0.03	-0.18	0.02	-0.29	0.08	1.49E-08	0.08	0.08	3.23E-01	-0.06	0.08	4.74E-01	-0.32	0.08	7.78E-05
Lac	rs12945978	17	633567	*FAM57A*, upstream gene variant	G/A	0.16	-0.17	0.03	-0.17	0.03	2.81E-08	0.08	0.03	3.51E-03	-0.09	0.03	2.28E-03	-0.16	0.03	1.37E-07
Gp	rs61876100	10	121189420	*GRK5*, intronic	GT	0.06	0.13	0.02	0.15	0.05	3.73E-08	0.02	0.05	6.50E-01	0.12	0.05	2.18E-02	0.15	0.05	5.21E-03
Val	rs118009109	22	28149774	MN1, intronic	C/T	0.08	-0.18	0.05	-0.21	0.05	4.00E-08	0.07	0.05	1.06E-01	-0.14	0.05	2.85E-03	-0.24	0.05	4.68E-07
SHDLTG	rs117796827	7	145658808	*CNTNAP2*, intergenic	T/C	0.02	0.22	0.03	0.29	0.10	4.00E-08	0.07	0.10	4.95E-01	0.27	0.10	6.95E-03	0.30	0.10	2.98E-03
Tyr	rs72676787	14	33614549	*NPAS3*, intronic	C/T	0.02	-0.34	0.08	-0.34	0.09	4.09E-08	0.25	0.08	2.25E-03	-0.10	0.09	2.52E-01	-0.34	0.09	1.09E-04
His	rs73292433	12	11942525	*ETV6*, intronic	G/C	0.28	0.19	0.03	0.15	0.03	4.22E-08	-0.09	0.03	3.09E-03	0.08	0.03	9.60E-03	0.15	0.03	1.84E-06
MHDLTG	rs66799104	3	77312067	*ROBO2*, intronic	G/T	0.11	-0.09	0.01	-0.18	0.03	4.58E-08	0.05	0.03	1.36E-01	-0.02	0.03	5.39E-01	-0.19	0.03	6.96E-08

SNP: single nucleotide polymorphism; Chr: chromosome; POS: position; EA/NEA: effect allele (coding allele)/non-effect allele (non-coding) allele; EAF: effect allele frequency/coding allele frequency; beta: effect size per coding allele; se: standard error.

§ the response to a liquid meal expressed as % change from the fasting state, calculated by using the raw OrNLS residuals (i.e. without inverse rank normal) divided by the baseline fasting measurements times 100, which gives the % change per allele.

Figure 1. Analysis workflow.

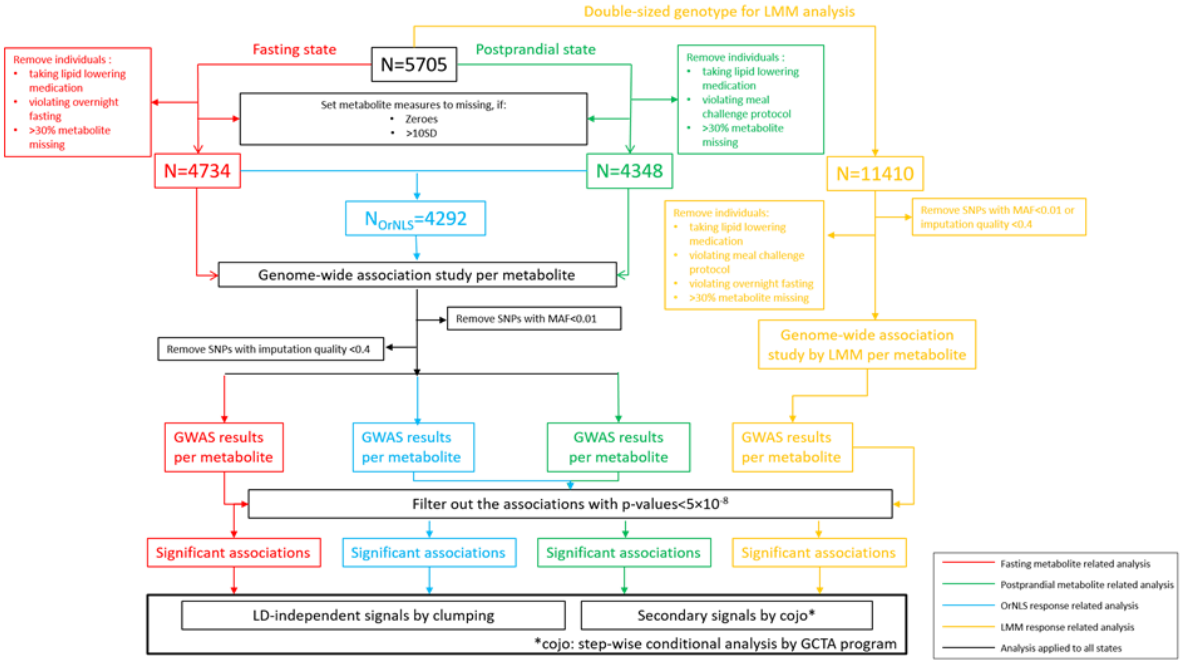

Figure 2. Miami plot of 148 fasting (top) and postprandial (bottom) metabolites.

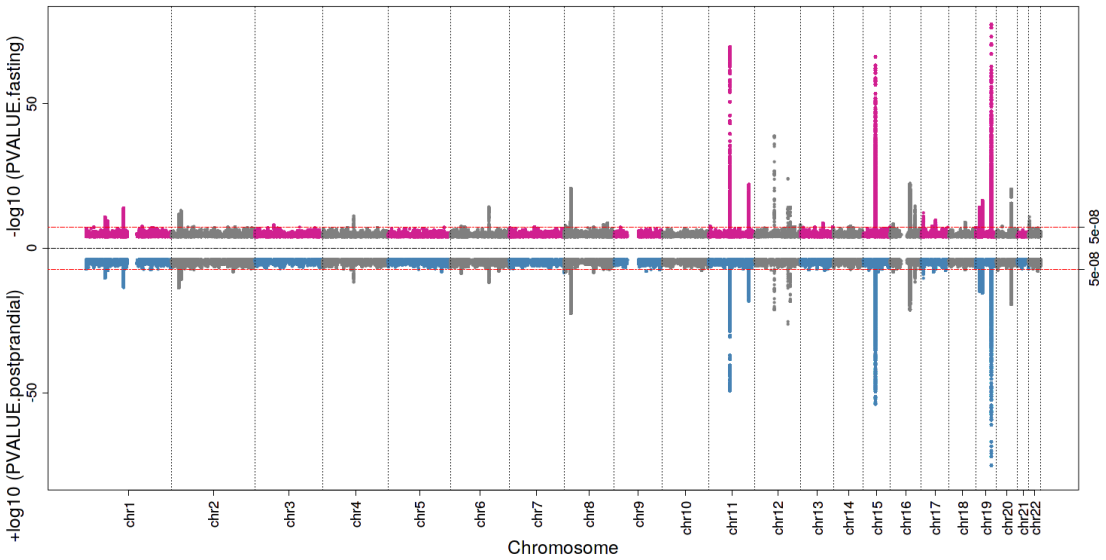

Figure 3. Combinatorial plot of glucose response signals identified from GWAS. (a). Regional plot for the lead signal rs10830963; (b). An interaction plot of clinical chemistry glucose levels at fasting, 30 minutes and 150 minutes after a liquid mixed meal in the NEO cohort, stratified by rs10830963 genotype; (c). The -log10(p-values) of associations between rs10830963 and several glycaemic traits from literature; FG: fasting glucose; BW: birth weight; HbA1c: Hemoglobin A1C (HbA1c) test; T2D: type 2 diabetes; IResadjISI: corrected insulin response adjusted for insulin sensitivity index; IDI: Insulin disposition index; HOMA-B: the Homeostasis Model Assessment (HOMA) estimates steady state beta cell function (%B).

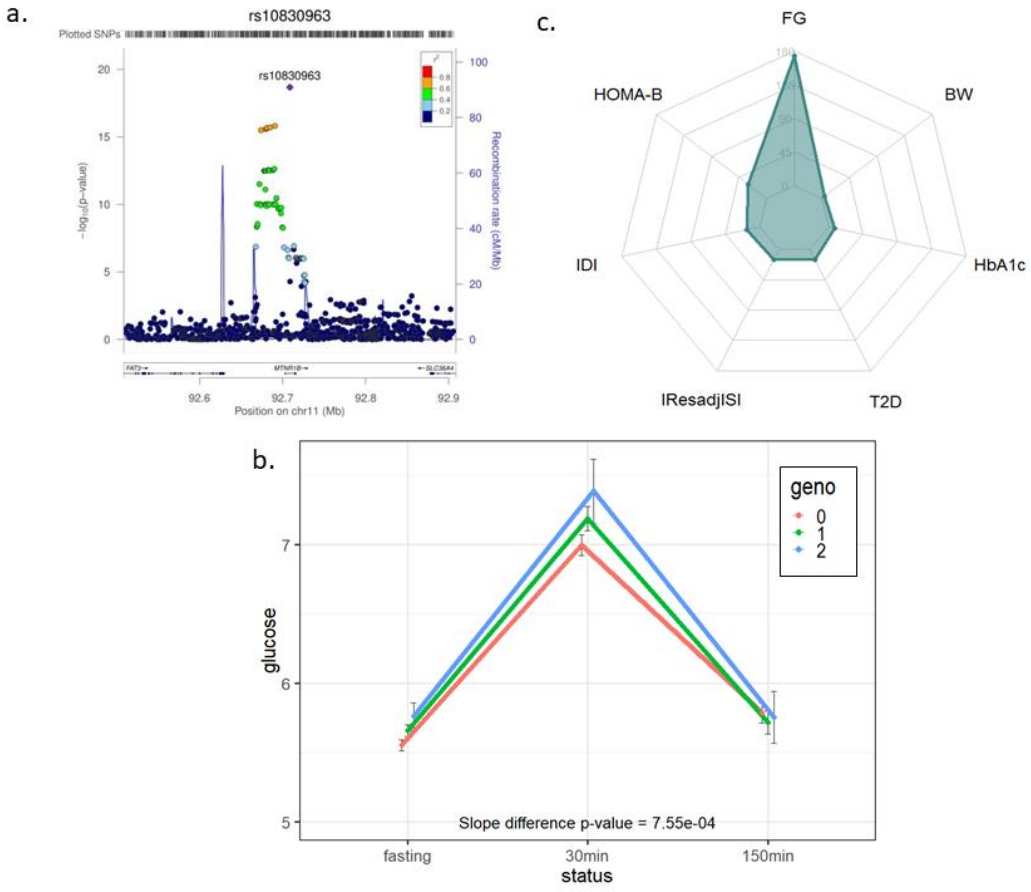

Figure 4. Pathophysiological insights from novel chylomicrons and extremely large VLDL particle associations. (a). The eQTL of three SNPs (rs458741, rs467022, rs173964) located in the *ANKRD55* locus that are associated with chylomicrons and extremely large VLDL particle response to a liquid mixed meal (response[r]XXLVLDLC, rXXLVLDLCE, rXXLVLDLL, rXXLVLDLP, rXXLVLDLTG, rXXLVLDLFC, rXXLVLDLPL), and significant traits that are associated with three SNPs located in the *ANKRD55* locus by phenome wide association studies (Phewas) in the UKBiobank. (b). The associations between rs173964 and type 2 diabetes as well as insulin sensitivity index in the NEO study and from literature, combined with an illustrations of lipid absorption and transport. ER: endoplasmic reticulum.

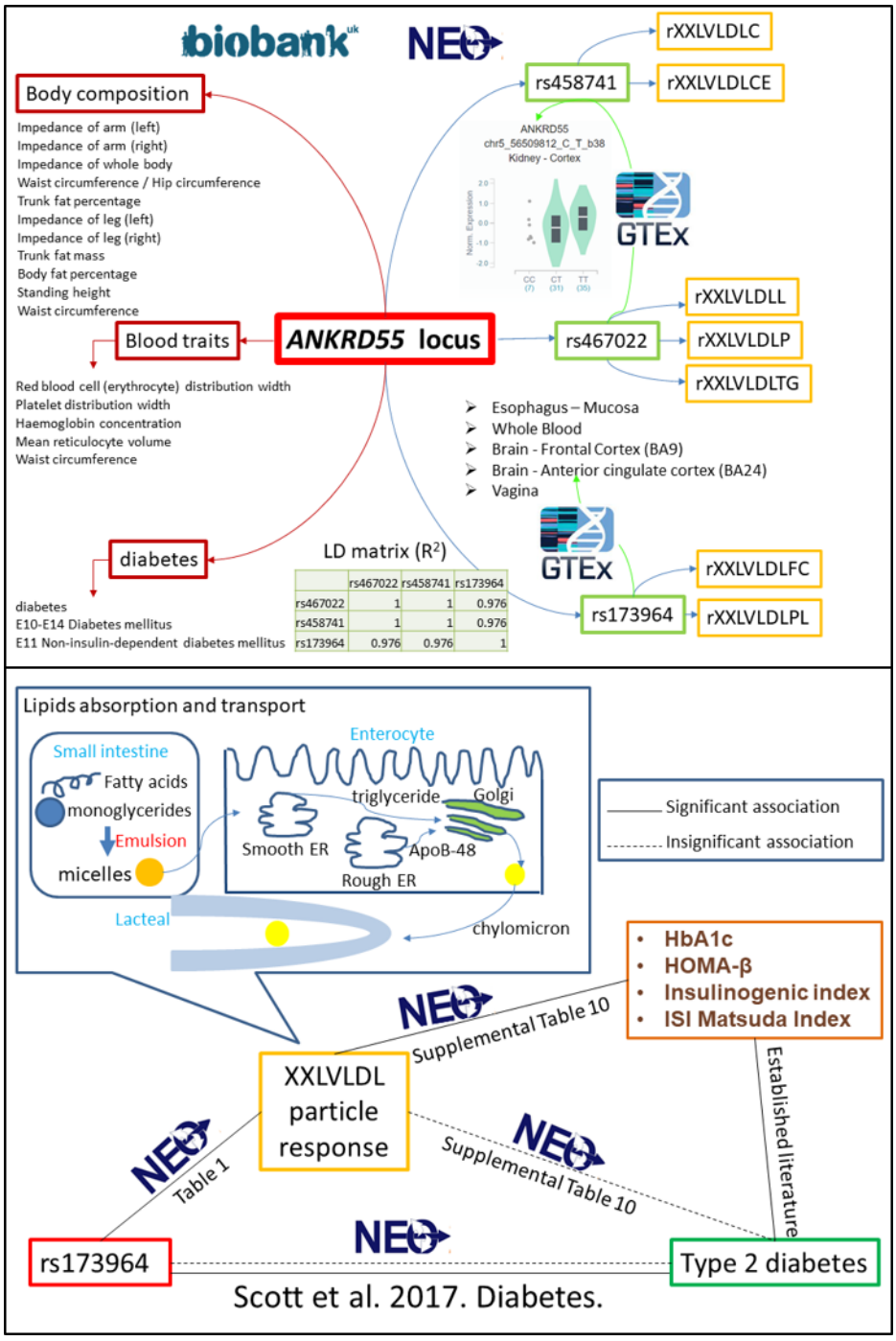

Figure 5. Heritability of 148 metabolites on different fasting status.